FULL PAPER

# Hyaluronic Acid Catechol: A Biopolymer Exhibiting a pH-Dependent Adhesive or Cohesive Property for Human Neural Stem Cell Engineering

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**Nature has developed materials that are integrated and effective at controlling their properties of adhesiveness and cohesiveness; the chemistry of these materials has been optimized during evolution. For example, a catechol moiety found in the adhesive proteins of marine mussels regulates its properties between adhesion and cohesion, rapidly adapting to environmental conditions. However, in synthetic materials chemistry, introduced chemical moieties are usually monofunctional, either being adhesive or cohesive; typically, this is not effective compared to natural materials. Herein, it is demonstrated that hyaluronic acid-catechol (HA-catechol) conjugates can exhibit either adhesiveness, functionalizing the surface of materials, or cohesiveness, building 3D hydrogels. Up to now, catechol-conjugated polymers have shown to be useful in one of these two functions. The usefulness of the polymer in stem cell engineering is demonstrated. A platform for neural stem cell culture may be prepared, utilizing the adhesive property of HA-catechol, and hydrogels are fabricated to encapsulate the neural stem cells, utilizing the cohesive property of the HA conjugate. Moreover, the HA-catechol hydrogels are highly neural stem cell compatible, showing better viability compared to existing methods based on HA hydrogels.**

## 1. Introduction

The introduction of adhesive or cohesive properties to materials is important to create functional materials, particularly in biomaterial science. The surface immobilization of biopolymers such as poly(ethylene glycol) (PEG), heparin, or hyaluronic acid (HA) has become increasingly critical in a variety of biomedical applications, such as for controlling the stability and performance of nanoparticles through surface-immobilized molecules,[1] for uncovering cellular functions and mechanisms through biointerfaces created by tethering biologically inert and/or active molecules on solid substrates,[2] and for engineering the surfaces of medical devices in contact with the blood (such as cardiovascular stents and valves) to inhibit re-endothelialization or calcification.[3] Other widely known examples of utilizing adhesive properties include medical adhesives,[4] the creation of anti-coagulant immobilized surfaces,[5] and substrate-independent layer-by-layer assembly.[6]

Cohesiveness has been used to prepare three-dimensional biomaterials such as hydrogels. Cohesive properties can be achieved either by intermolecular associations, as shown in the triblock-copolymers[7] and DNA,[8] or by chemical reactions between amine and carboxylic acid groups.[9] Materials prepared by cohesive mechanisms have been applied to tissue-engineering scaffolds[10] and depots for drug delivery.[11] Typically, however, when a functional biopolymer is designed, it tends to exhibit either adhesiveness or cohesiveness. In case of HA, for example, hydrogels (i.e., cohesiveness) have been prepared by partial chemical tethering of thiol-, aldehyde-, dihydrazide-, and methacrylate-containing moieties.[12] For surface immobilization (i.e., adhesiveness), different strategies have been used.[13]

Biomaterials in nature use an integrated, effective approach to control their properties of adhesiveness and cohesiveness. A catechol moiety found in the proteins of marine mussels, *Mytilus edulis,* plays dual adhesive and cohesive roles.[14] The molecular basis for adhesion is the reversible coordination of metal oxides, $\pi$–$\pi$ interactions with various synthetic polymers, and irreversible covalent bond formation with natural organic

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surfaces.[15] For cohesive function, catechol undergoes pH-dependent oxidative reactions by the DOPA-to-quinone transition.[16] Therefore, we hypothesized that catechol is a reasonable bioinspired candidate for the design of a biopolymer that can function as both an adhesive and cohesive material depending on the external environment. Herein, we successfully demonstrated for the first time that a widely used biopolymer, HA, conjugated to catechol (HA-catechol) exhibits both adhesive and cohesive properties depending on the external environment. One parameter determining the adhesive or cohesive properties is the pH: acidic solutions drive HA-catechols to be adhesive, resulting in surface coating, whereas basic conditions increase its cohesive property, resulting in hydrogels. So far, a variety of catechol-conjugated biopolymers, such as PEG-catechol, heparin-catechol, polyethylenimine-catechol, and HA-catechol, have been reported. As previously mentioned, however, catechol-conjugated polymers designed specifically for surface adhesion does not have effective molecular configurations for hydrogel preparation, which has been demonstrated in the cases of linear PEG-catechols,[17] heparin-catechol,[5] polyethylenimine-catechol,[6,18] and the previous HA-catechol design.[19] In contrast, multi-arm PEG-catechols (despite their use of similar catechol moieties) have been used successfully as molecules for hydrogel preparation, but not for adhesive surface functionalization.[20] In particular, polymers with a catechol-tethered configuration, such as the HA-catechol used in this study, have not been successfully used in hydrogel preparation thus far.

The adhesive and cohesive properties of the HA-catechol can be applied to the development of functional 2D substrates for the culture and 3D scaffolds for transplantation of stem cells on demand. Several surface coating materials and hydrogels have been reported for the culture and transplantation of human neural stem cells (hNSCs) previously. For example, the coating of cationic polymers such as poly(L-lysine) and poly(L-ornithine) has been used to improve the adhesion and differentiation of NSCs.[21,22] However, the methods typically result in a marginal effect on enhancement of NSC culture because the surface-functionalized polymers are biologically inactive, lack adhesive property on substrates, or exhibit a certain degree of cytotoxicity.[23] Hydrogels have also been prepared utilizing several polymers (e.g., HA, PEG, etc.) for NSC encapsulation.[24] However, most approaches for hydrogel preparations have been photopolymerization, in which the use of ultraviolet light for crosslinking results in an adverse effect on cell viability.[25] Matrigel has been a widely used material for the culture and transplantation of NSCs,[26] but concerns related to immunogenicity and pathogen transmissions due to the chemically undefined nature of Matrigel have triggered researchers to develop chemically defined substrates or scaffolds.[27]

Our study also demonstrated that substrates and hydrogel scaffolds for hNSCs can be easily prepared with HA-catechol, through its pH-dependent property, to be adhesive or cohesive. Owing to the specific interaction between HA and CD44 (HA receptor) on NSCs,[28] hNSCs could be easily cultured on a two-dimensional substrate after one-step functionalization by HA-catechols. Stable culturing of hNSCs onto various substrates has been a challenging task because of problems related to cell adhesion on substrates. Furthermore, hNSCs encapsulated within HA-catechol hydrogels were shown to exhibit higher cell viability (greater than 80%) after 4 d of culture, compared to those within an HA hydrogel prepared by conventional UV crosslinking (viability less than 50% after 4 d).

## 2. Results and Discussion

To prepare the HA-catechol conjugate, dopamine was coupled to the carboxyl group of HA by the EDC coupling reaction (**Figure 1**A). The reaction was performed in an aqueous buffer for 9 h, and the reaction pH was maintained at around 6. The content of catechol was determined by ultraviolet-visible (UV-Vis) spectroscopy at 280 nm using dopamine standard solutions, with results showing that approximately 5.1 ± 0.4% ($n$ = 3) of the carboxylic acid groups in the HA chain were conjugated with dopamine. The single absorbance peak appeared at 280 nm, and the absence of additional peaks at wavelengths longer than 300 nm showed that the conjugated catechol was not oxidized (Figure 1B).

To characterize surfaces, substrates (Au and $TiO_2$) were immersed in the HA-catechol solution at an acidic pH (pH < 2) for 1 h and subsequently washed with DDW (Figure 2A). We characterized the functionalized surface using reflective Fourier transform infrared (FT-IR) spectroscopy and X-ray photoelectron spectroscopy (XPS). The FT-IR spectra for the HA-catechol-coated $TiO_2$ surface demonstrate the presence of HA-catechol on the surface (background FT-IR signals were taken by using unmodified $TiO_2$). The various constituents were observed at the following frequencies: methylene ($–CH_2$) at 2940–2920 $cm^{-1}$ (m) and 2860–2850 $cm^{-1}$ (m), amide bonds through C=O stretching at 1700–1250 $cm^{-1}$ (vs), –COO– salts of carboxylic acid at 1650–1500 $cm^{-1}$ (vs), NH bending at 1560–1530 $cm^{-1}$ (vs), and ethers (–C–O–C–) at 1100 $cm^{-1}$ (vs). Hydroxyl groups showed the primary –OH stretch at 3640–3630 $cm^{-1}$ (s) and the general –OH stretch at 3350–3250 $cm^{-1}$ (s) (**Figure 2**B). Detection of nitrogen and amide by FT-IR spectroscopy indicated successful HA-catechol surface coating. High-resolution C1s data from the XPS

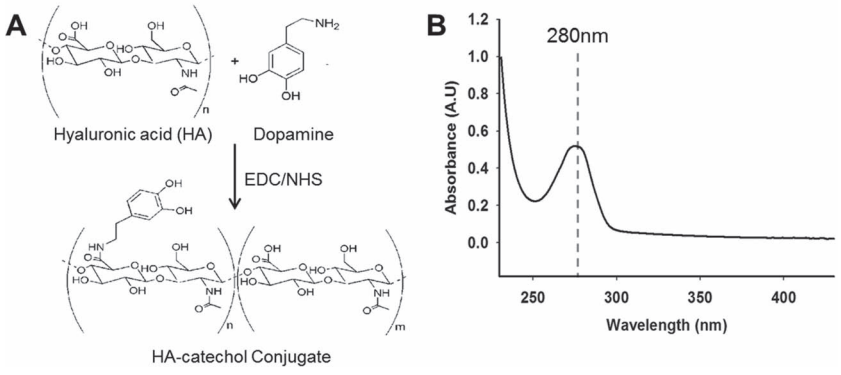


**Figure 1.** A) A schematic representation of preparing HA-catechol conjugates. B) UV-Vis spectra of the HA-catechol conjugate, with a peak appearing at 280 nm. The lack of detection of a peak after 300 nm shows that the conjugated catechol was not oxidized.

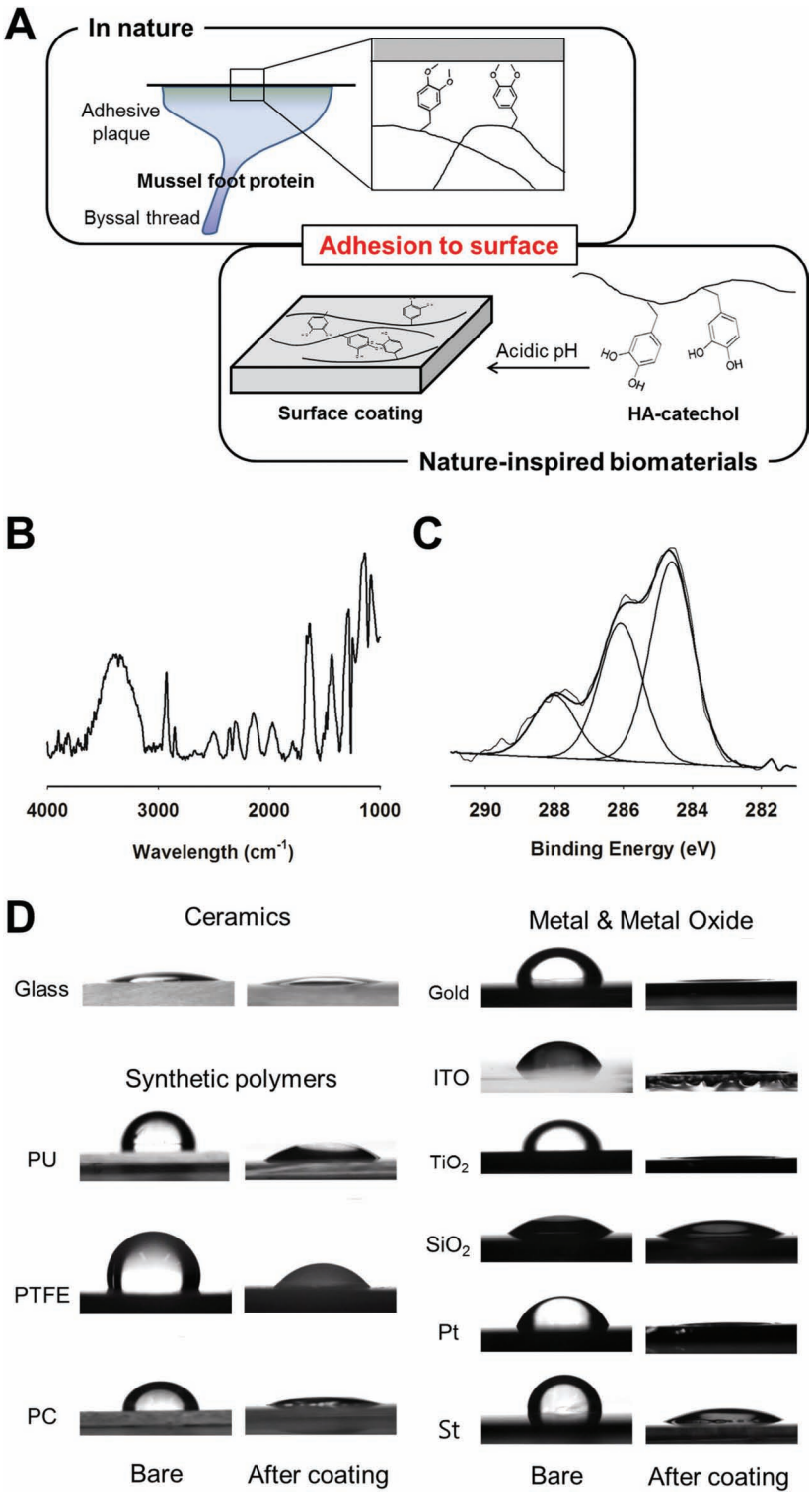


**Figure 2.** A) A schematic representation of the adhesive function of catechol in nature (top), as well as an in vitro condition (bottom), indicating that the HA-catechol conjugate is adhesive to a variety of surface chemistries under acidic conditions. Characterization of the surface functionalized by the HA-catechol: B) FT-IR spectra of the $TiO_2$ surface coated by the HA-catechol; C) XPS data (high-resolution C1s) of the $TiO_2$ surface coated by the HA-catechol; and D) measurement of static contact angles for substrates before and after the coating by the HA-catechol. HA-catechol is adhesive to a variety of substrates, including ceramics (glass), synthetic polymers (PU, PTFE, PC), metals (gold, Pt, stainless steel (St)), and metal oxides ($TiO_2$, $SiO_2$, ITO).

analysis of the HA-catechol-coated Au surface confirmed successful HA-catechol surface coating, in which 286 eV for C–O and 288 eV for C=O represented functional groups of HA on the surface (Figure 2C).

The synthesized HA-catechol exhibited a remarkable adhesive property on a variety of substrates under acidic conditions (pH < 2). Goniometric measurement is a convenient tool to characterize surface modifications by HA-catechol. After being coated, the surface becomes hydrophilic, exhibiting a static contact angle of less than 10°. The adhesive property allowed facile surface modifications of glass (14.7° ± 2.2° → < 10°), gold (Au) (69.9° ± 1.1° → ≈0°), platinum (Pt) (64.6° ± 3.0° → ≈0°), stainless steel (St) (91.4° ± 1.9° → 18.3° ± 2.9°), titanium oxide ($TiO_2$) (72.2° ± 1.6° → ≈0°), silicon oxide ($SiO_2$) (32.7° ± 0.8° → 20.6° ± 2.1°), indium-tin oxide (ITO) (56.6° ± 1.2° → ≈0°), poly(urethane) (PU) (72.4° ± 1.8° → 29.1° ± 5.2°), poly(tetrafluroethylene) (PTFE) (99.7° ± 5.0° → 42.9° ± 3.0°), and poly(carbonate) (PC) (70.3° ± 6.7° → 15.3° ± 2.1°) (Figure 2D). It is noteworthy that most functionalized surfaces showed very hydrophilic properties (less than 10°), and some substrates, such as glass, Au, Pt, $TiO_2$, and ITO, exhibited superhydrophilic properties, indicating nearly 100% coverage by the immobilized HAs. In cases of St, $SiO_2$, and PC, the contact angle values are between 10° and 20°, indicating almost 100% surface coverage with some extent of coating defects. In cases of PTFE and PU, the static contact angle was higher than 20°. These results might represent partial surface modifications. Changes in the concentration and the coating time (1 h in this study) of HA-catechol might increase the surface coverage. As there are a number of methods for HA surface immobilizations, it would be reasonable to discuss the coating stability when using catechol as a surface anchor moiety. Immobilization of HAs by using catechol was shown to be stable compared to the widely used thiol-gold interactions.[19b] In this study, the thiolated HA bound onto the gold surfaces was rapidly dissociated, but the end-functionalized HA-catechol stably remained on the same surfaces.

In addition, the HA-catechol conjugate becomes a cohesive polymer when dissolved in an alkaline buffer (pH 8 to 9). In the presence of an oxidizing agent, sodium periodate ($NaIO_4$), the polymer solution immediately became a hydrogel by the chemical crosslinking between the conjugated catechol moieties (**Figure 3**A). Inversion of the HA-catechol solution indicates whether its physical state is sol or gel, and we found that fast gelation was observed

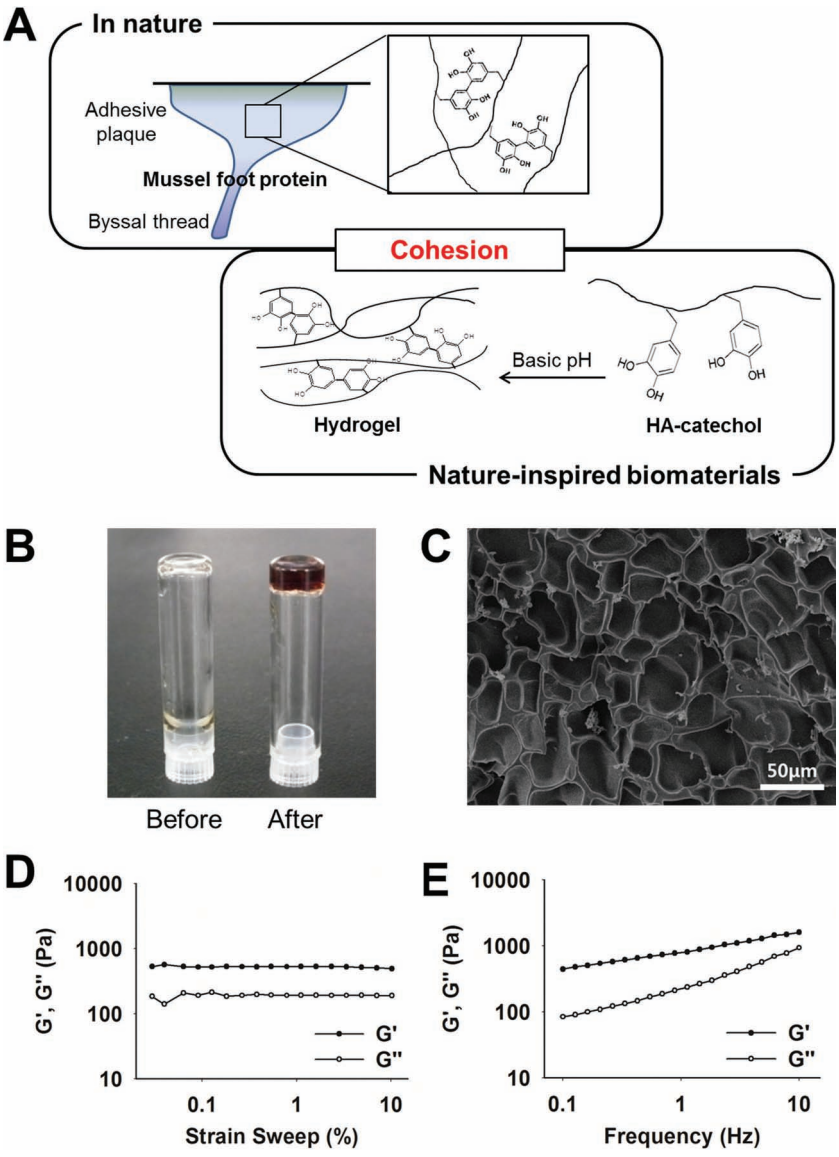


**Figure 3.** A) A schematic representation of the cohesive function of catechol in nature (top) and in vitro (bottom), indicating that the HA-catechol conjugate forms hydrogels by intermolecular catechol–catechol reactions (i.e., cohesiveness) in alkaline conditions. Physical properties of the hydrogel formed by the HA-catechol: B) a photograph of the HA-catechol hydrogel; C) an SEM image of the lyophilized hydrogel, the average pore size was 30 μm; and D,E) mechanical properties as investigated by rheometeric analysis through a strain sweep (D) and a frequency sweep (E).

when the $NaIO_4$:catechol stoichiometric ratio was 1.5:1. In this condition, HA-catechol became a gel within 10 s (Figure 3B). The cohesive mechanism has been well demonstrated in PEG hydrogel cases,[20] in which the deprotonation of the hydroxyl groups in catechol results in quinone formation,[29] resulting in cohesive crosslinking reactions. Unlike the previous PEG-catechol hydrogel reports in which branched, multi-arm PEGs were used to form hydrogels, hydrogel formation utilizing the HA-catechol conjugate has been challenging because of the linear chain configuration of HAs.

The pore size of the hydrogel was analyzed by scanning electron microscopy (SEM) after removing its water content by lyophilization (Figure 3C). The average pore size was measured at 30 μm, and we believe that the size becomes larger when hydrated, making the hydrogel suitable for cell encapsulation. The mechanical properties were investigated by rheology analysis. We measured strain sweep and frequency sweep after the gel was formed in the presence of 1.5 equivalents of $NaIO_4$ under basic conditions (Figure 3D,E). The storage modulus ($G'$) was approximately 550 Pa, and the loss modulus ($G''$) was approximately 200 Pa. The strain-sweep data revealed that the moduli were independent of the strain range, demonstrating that the hydrogel was stably formed (Figure 3D). However, the moduli appeared to increase gradually over time in the frequency sweeping experiment (Figure 3E). This result might have been due either to the unreacted catechol moieties along the HA chains, which may have reacted further during the mechanical test, or to reversible bonds, such as hydrogen bonding, which result in variations in the relaxation of HA chains. In general, polymers with a high molecular weight often require significant time to rearrange themselves to have a free-energy minimum. This effect can be observed at a high-frequency conditions in which the chain rearrangement might not be completed in a short timescale (i.e., a high frequency sweep). Therefore, "solid-like" behavior is often detected in this condition.[30] Although the mechanical strength is relatively low, the hydrogel could be applied as a scaffold for tissue engineering and is particularly suitable for soft tissue, such as neuronal tissue.

The adhesive property of the HA-catechol conjugate allowed us to prepare platforms for hNSC culture with ease. A variety of substrates made of synthetic polymers, poly(styrene) (PS), poly(lactic-*co*-glycolic acid) (PLGA), poly(dimethylsiloxane) (PDMS), or metals (Si and Ti) were coated with HA-catechol (HA molecular weight: 50, 200, and 700 kDa) at a pH of 2.0 and were then used for the hNSC culture in the absence of mitogenic factors (bFGF and LIF). NSCs usually undergo spontaneous differentiation without the supplementation of mitogenic factors.[21] The coating of HA-catechol significantly facilitated the adhesion of hNSCs onto the substrates made of various polymers and metals (**Figure 4** and Figure S1 in the Supporting Information), but on the non-coated substrates the hNSCs did not adhere tightly and spread over the substrates (Figure 4). Most hNSCs that adhered onto the HA-catechol-coated substrates remained viable, but a number of dead cells were observed on the non-coated substrates (Figure 4), most likely due to anoikis, a form of cell death caused by inefficient adherence onto substrates or matrices. Furthermore, the hNSCs on the HA-catechol-coated

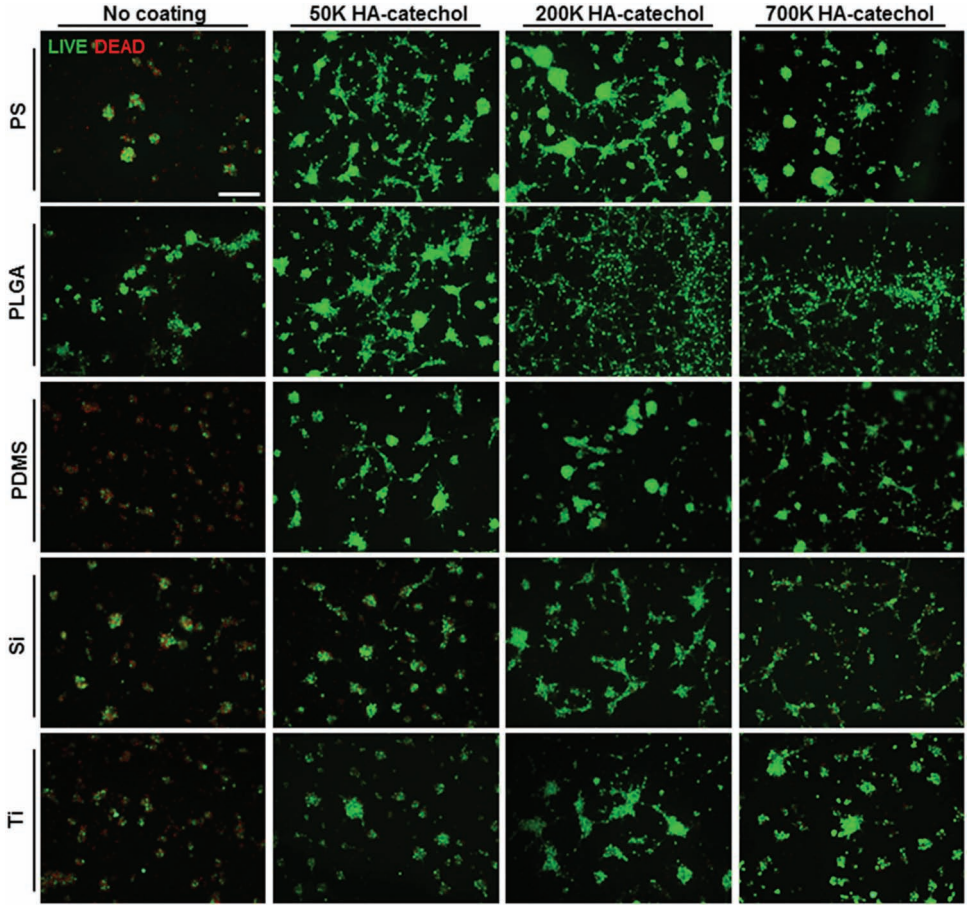


**Figure 4.** Live/dead staining of the cultured hNSCs onto the HA-catechol-functionalized substrates or onto the uncoated substrates 3 d after seeding (HA molecular weights: 50, 200, and 700 kDa). The substrates for HA-catechol coating include three types of synthetic polymers (PS, PLGA, and PDMS) and two metals (Si and Ti). Live cells are labeled with green fluorescent staining, while red staining indicates dead cells. The scale bar indicates 200 μm.

substrates exhibited neurite extension (Figure 4), indicating enhanced differentiation of hNSCs. Because NSCs express the surface receptor (CD44) for HA, the HA-catechol substrate coating is thought to improve NSC adhesion.[28] These results demonstrate that HA modified with catechol may be useful for the culture of hNSCs for therapeutic purposes.

Cohesive hydrogels prepared from HA-catechol under alkaline pH conditions were tested for the 3D culture of hNSCs. The viability of hNSCs in HA-catechol hydrogel was compared with that in HA-methacrylate hydrogel. The crosslinking of methacrylate conjugated to the HA backbone is known to be one of the conventional techniques for HA hydrogel formation.[12] Live/dead staining revealed that most NSCs were viable in the catechol-modified HA hydrogel 7 d after culture (Figure S2, Supporting Information). The crosslinking of HA-catechol using an oxidizing agent, sodium periodate ($NaIO_4$), did not exhibit cytotoxicity during the cell encapsulation (**Figure 5** and Figure S2). In contrast, the viability of hNSCs encapsulated in the HA-methacrylate hydrogel decreased, most likely due to the use of radical-generating photoinitiator (Irgacure 2959) and exposure to ultraviolet light for the crosslinking of methacrylate (Figure 5 and Figure S2). A significant population of hNSCs was stained as dead cells 7 d after culture in the HA-methacrylate hydrogel

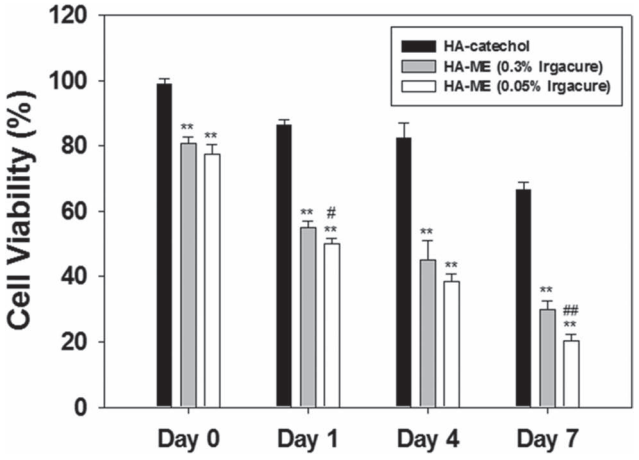


**Figure 5.** The viability of hNSCs encapsulated in HA-catechol hydrogels. Live/dead staining of hNSCs in HA-catechol hydrogel and HA-methacrylate (HA-ME) hydrogel (photoinitiator concentration; 0.05% and 0.3%) was performed days 0, 1, 4, and 7 after cell encapsulation. The viability of hNSCs encapsulated in the HA-catechol and HA-methacrylate (HA-ME) hydrogels was quantified by investigating the percentage ratio of live cells to total cell population in the live/dead stained images ($n$ = 3; $^{**}p < 0.01$, for the HA-catechol group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ for the HA-methacrylate group with 0.3% Irgacure).

(Figure 5). The viability of hNSCs was two-fold higher in the HA-catechol hydrogel (approximately 70%) than in the HA-methacrylate hydrogel (less than 30%) 7 d after culture (Figure 5). Our data show that the HA-catechol hydrogel could induce efficient adhesion of hNSCs and support a high level of hNSC viability in a 3D environment. Thus, HA-catechol hydrogel would be able to provide functional 3D scaffolds for hNSC transplantation.

## 3. Conclusions

We have demonstrated that the mussel-inspired HA-catechol conjugate shows both adhesive and cohesive properties, which are dictated by the external pH conditions. The property regulation between adhesion and cohesion demonstrated in this study is the first manifestation of a biopolymer that truly mimics the dual properties shown in mussel adhesive proteins. A biopolymer with dual adhesive and cohesive properties can be considered a new concept in materials science because, thus far, researchers have adopted different chemical strategies in polymer design, targeted specifically toward either adhesion or cohesion. With the utilization of biospecific interactions between HA and hNSCs, the previously challenging goals of stable two-dimensional culture and hNSC encapsulation within gels become straightforward tasks that result in better hNSC stability and viability than with the existing methods.

## 4. Experimental Section

*Materials*: Hyaluronic acid (HA, MW = 130 kDa) was purchased from Lifecore Co (Chaska, MN, USA). Sodium periodate ($NaIO_4$), albumin, fluorescein isothiocyanate conjugate bovine (FITC-BSA), dopamine hydrochloride, *N*-hydroxy succinimide (NHS) and hyaluronidase from bovine testes (850 units $mg^{-1}$) were purchased from Sigma-Aldrich (St. Louis, MO). 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (coupling agent for peptides synthesis, EDC) from TCI-SU (Tokyo, Japan) was used.

*Synthesis of HA-Catechol Derivative*: HA (1 g, MW = 130 kDa) was dissolved in degassed 50 mL of 2× phosphate buffered saline (PBS). EDC (485.3 mg) and NHS (291.4 mg) was slowly added to the mixed solution, until the final molar ratio of HA/EDC/NHS was set to 1:1:1. After 20 min stirring, 0.5 g of dopamine was added to the mixture. The pH value of the solution was continuously monitored and maintained from 4 to 6 for 9 h. After the reaction, the solution was purified by dialysis (MWCO = 14 000) for 2 d in 2× PBS and acidified DDW (pH < 2) and was subsequently lyophilized, which resulted in a white powder. The content of catechol was confirmed by ultraviolet-visible spectroscopy measuring absorbance at 280 nm, demonstrating that about 5.1 ± 0.36% of hyaluronic acid was conjugated with catechol. The quantitative measurement was performed by UV-Vis spectrophotometer with a dopamine standard.

*Surface Coating on a Variety of Substrates*: An HA-catechol derivative was dissolved in 1× PBS at a 2% (w/v) concentration; sodium periodate ($NaIO_4$), an oxidizing agent, was added at a ratio of 1.5 mol to 1 mol of catechol. After lowering the solution pH to below 2, the prepared substrates were coated in the solution for 1 h. Contact angle measurement performed before and after the coating confirmed a change in the hydrophilicity of the surface, indicating that the coating had been successfully performed. Contact-angle measurements were performed with a Phoenix 300 goniometer (Surface Electro Optics Co., Ltd., Korea). Contact angles were measured using a 20 μL water droplet.

*Formation of HA-Catechol Hydrogels*: HA-catechol hydrogels were formed by the crosslinking of catechols. First, HA-catechol was dissolved in 1× PBS, 10% (w/v). The pH of the solution was adjusted from 8 to 9 by adding NaOH. When $NaIO_4$ (at a ratio of 1.5 mol to 1 mol of catechol) was added (0.4 mg/100 μL of HA-C), HA-catechol hydrogel was spontaneously synthesized without any further treatment. The gelation time of the hydrogel was measured while varying the amount of sodium periodate (from 1.0 to 2.2 equivalents to the molar amount of catechol. The gelation time was determined when the solution flow stopped in an inverted vial.

*Oscillatory Rheometry and SEM Analysis*: Oscillatory rheometry was performed with a rotating rheometer (Bohlin Advanced Rheometer with a parallel 20 mm plate, Malvern Instruments, UK) to determine the mechanical properties of the HA-catechol hydrogels. A strain sweep was performed at a strain of 10%, and a frequency sweep was performed at 0.1–10 Hz, 10 min after the gel formation. SEM images were obtained after the hydrogel lyophilization using the S-4800 field emission microscope (HITACHI, Japan) with an accelerating voltage of 15 keV.

*Culture of Human Neural Stem Cells (hNSCs)*: Human fetal NSCs were derived from the telencephalon (HFT13), as previously described.[31] The cells were plated at a cell density of $6.0 \times 10^5$ $mL^{-1}$. Human NSCs were cultured in Dulbecco's Modified Eagle Medium (nutrient mixture F-12) (DMEM/F12) medium (Gibco BRL, Gaithersburg, MD, USA) supplemented with basic fibroblast growth factor (bFGF, 20 ng $mL^{-1}$; Sigma), leukemia inhibitory factor (LIF, 10 ng $mL^{-1}$; Sigma), and N-2 supplement (Gibco BRL)) in humidified air with 5% $CO_2$ at 37 °C.

*Cell Adhesion Test*: The adhesion of hNSCs was examined on five types of substrates (synthetic polymers: PS, PLGA, and PDMS; metals: Si and Ti) coated with HA-catechol (HA molecular weight: 50, 200, and 700 kDa). The coating condition employed a 1 h incubation of 1% (w/v) HA-catechol with 2 equivalents (molar ratio) of $NaIO_4$ in PBS at pH 2.0. Human NSCs ($2.0 \times 10^5$ cells per sample) were seeded onto the HA-catechol-coated substrates and cultured without the supplementation of mitogenic factors. Three days later, the adhesion and viability of hNSCs was examined by staining the cells with the live/dead assay kit (Invitrogen) using double staining of calcein AM and ethidium homodimer under a fluorescent microscope (Olympus IX71). Calcein AM stains the cytoplasm of viable cells green, and the ethidium homodimer stains the nuclei of non-viable cells red. The bare substrates without HA-catechol coating served as controls. The area of NSC adhesion on the substrates was quantified using Image J software from the NIH.

*Cell Encapsulation Test*: Human NSCs ($1.0 \times 10^6$ cells per 450 μL of gel solution) were encapsulated in HA-catechol or HA-methacrylate hydrogel (concentration: 2% (w/v), HA molecular weight: 200 kDa). The HA-catechol hydrogel was formed by crosslinking with sodium periodate (4.5 mg $mL^{-1}$ $NaIO_4$, Sigma) and sodium hydroxide (0.4 M NaOH, Sigma). To form the HA-methacrylate hydrogel, HA-methacrylate solution was supplemented with a photoinitiator (0.05% and 0.3% Irgacure 2959 in PBS, I2959 Ciba Specialty Chemicals, Basel, Switzerland) and exposed to ultraviolet light (average intensity: 10 mW $cm^{-2}$, UVP, Upland, CA) for 2 min (0.3% Irgacure 2959) or 10 min (0.05% Irgacure 2959). The viability of hNSCs in the HA-catechol hydrogel was examined by the live/dead staining kit (Invitrogen) at several time points after encapsulation (days 0, 1, 4, and 7) and compared with the hNSC viability in the HA-methacrylate hydrogel. The stained cells were observed with a fluorescence microscope (Olympus IX71), and the cell viability was quantified by determining the ratio of live cells to the total cell populations.

## Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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[1] a) H. Chung, H. Lee, K. Bae, Y. Lee, J. Park, S. Cho, J. Hwang, H. Park, R. Langer, D. Anderson, T. Park, *ACS Nano* **2011**, *5*, 4329; b) J. Jokerst, T. Lobovkina, R. Zare, S. Gambhir, *Nanomedicine* **2011**, *6*, 715.
[2] C. Chen, M. Mrksich, S. Huang, G. Whitesides, D. Ingber, *Science* **1997**, *276*, 1425.
[3] S. Schopka, T. Schmid, C. Schmid, K. Lehle, *Materials* **2010**, *3*, 638.
[4] a) Y. Lee, H. Chung, S. Yeo, C. Ahn, H. Lee, P. Messersmith, T. Park, *Soft Matter* **2010**, *6*, 977; b) J. Ryu, Y. Lee, W. Kong, T. Kim, T. Park, H. Lee, *Biomacromolecules* **2011**, *12*, 2653.
[5] I. You, S. Kang, Y. Byun, H. Lee, *Bioconjugate Chem.* **2011**, *22*, 1264.
[6] H. Lee, Y. Lee, A. Statz, J. Rho, T. Park, P. Messersmith, *Adv. Mater.* **2008**, *20*, 1619.
[7] a) B. Jeong, Y. Bae, D. Lee, S. Kim, *Nature* **1997**, *388*, 860; b) B. Jeong, S. Kim, Y. Bae, *Adv. Drug Delivery Rev.* **2002**, *54*, 37.
[8] a) S. Um, J. Lee, N. Park, S. Kwon, C. Umbach, D. Luo, *Nat. Mater.* **2006**, *5*, 797; b) M. Campolongo, S. Tan, J. Xu, D. Luo, *Adv. Drug Delivery Rev.* **2010**, *62*, 606.
[9] a) J. Drury, D. Mooney, *Biomaterials* **2003**, *24*, 4337; b) A. Hoffman, *Adv. Drug Delivery Rev.* **2002**, *54*, 3; c) L. Yu, J. Ding, *Chem. Soc. Rev.* **2008**, *37*, 1473; d) S. Van Vlierberghe, P. Dubruel, E. Schacht, *Biomacromolecules* **2011**, *12*, 1387.
[10] a) R. Langer, J. Vacanti, *Science* **1993**, *260*, 920; b) B. Slaughter, S. Khurshid, O. Fisher, A. Khademhosseini, N. Peppas, *Adv. Mater.* **2009**, *21*, 3307; c) O. Fisher, B. Larson, P. Hill, D. Graupner, M. Nguyen-Kim, N. Kehr, L. De Cola, R. Langer, D. Anderson, *Adv. Mater.* **2012**, *24*, 3032.
[11] T. Hoare, D. Kohane, *Polymer* **2008**, *49*, 1993.
[12] J. Burdick, G. Prestwich, *Adv. Mater.* **2011**, *23*, H41.
[13] a) J. Zhang, B. Senger, D. Vautier, C. Picart, P. Schaaf, J. Voegel, P. Lavalle, *Biomaterials* **2005**, *26*, 3353; b) K. Suh, J. Yang, A. Khademhosseini, D. Berry, T. Tran, H. Park, R. Langer, *J. Biomed. Mater. Res. Part B* **2005**, *72*, 292; c) K. Sengupta, J. Schilling, S. Marx, M. Fischer, A. Bacher, E. Sackmann, *Langmuir* **2003**, *19*, 1775.
[14] J. Waite, N. Andersen, S. Jewhurst, C. Sun, *J. Adhesion* **2005**, *81*, 297.
[15] H. Lee, N. Scherer, P. Messersmith, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 12999.
[16] a) L. Burzio, J. Waite, *Biochemistry* **2000**, *39*, 11147; b) J. Waite, *Integr. Comp. Biol.* **2002**, *42*, 1172.
[17] a) H. Lee, K. Lee, K. Pyo, S. Park, H. Lee, *Langmuir* **2010**, *26*, 3790; b) J. Dalsin, B. Hu, B. Lee, P. Messersmith, *J. Am. Chem. Soc.* **2003**, *125*, 4253.
[18] E. Kim, I. Song, S. Lee, J. Kim, H. Lee, J. Jang, *Angew. Chem. Int. Ed.* **2012**, *51*, 5598.
[19] a) Y. Lee, H. Lee, Y. Kim, J. Kim, T. Hyeon, H. Park, P. Messersmith, T. Park, *Adv. Mater.* **2008**, *20*, 4154; b) H. Lee, K. Lee, I. Kim, T. Park, *Adv. Funct. Mater.* **2009**, *19*, 1884.
[20] a) C. Brubaker, H. Kissler, L. Wang, D. Kaufman, P. Messersmith, *Biomaterials* **2010**, *31*, 420; b) B. Lee, J. Dalsin, P. Messersmith, *Biomacromolecules* **2002**, *3*, 1038.
[21] A. Chojnacki, S. Weiss, *Nat. Protoc.* **2008**, *3*, 935.
[22] L. Pan, Y. Ren, F. Cui, Q. Xu, *J. Neurosci. Res.* **2009**, *87*, 3207.
[23] a) D. Fischer, Y. Li, B. Ahlemeyer, J. Krieglstein, T. Kissel, *Biomaterials* **2003**, *24*, 1121; b) D. Morgan, V. Larvin, J. Pearson, *J. Cell Sci.* **1989**, *94*, 553.
[24] a) S. Seidlits, Z. Khaing, R. Petersen, J. Nickels, J. Vanscoy, J. Shear, C. Schmidt, *Biomaterials* **2010**, *31*, 3930; b) S. Hynes, L. Mcgregor, M. Rauch, E. Lavik, *J. Biomater. Sci., Polym. Ed.* **2007**, *18*, 1017.
[25] N. Fedorovich, M. Oudshoorn, D. van Geemen, W. Hennink, J. Alblas, W. Dhert, *Biomaterials* **2009**, *30*, 344.
[26] M. Uemura, M. Refaat, M. Shinoyama, H. Hayashi, N. Hashimoto, J. Takahashi, *J. Neurosci. Res.* **2010**, *88*, 542.
[27] A. Higuchi, Q. Ling, Y. Ko, Y. Chang, A. Umezawa, *Chem. Rev.* **2011**, *111*, 3021.
[28] a) C. Rampon, N. Weiss, C. Deboux, N. Chaverot, F. Miller, D. Buchet, H. Tricoire-Leignel, S. Cazaubon, A. Evercooren, P. Couraud, *Stem Cells* **2008**, *26*, 1673; b) P. Schwartz, P. Bryant, T. Fuja, H. Su, D. O'Dowd, H. Klassen, *J. Neurosci. Res.* **2003**, *74*, 838.
[29] B. Liu, L. Burdine, T. Kodadek, *J. Am. Chem. Soc.* **2006**, *128*, 15228.
[30] a) K.-W. Song, G.-S. Chang, *J. Korean Pharm. Sci.* **2006**, *36*, 31; b) K. Ghosh, X. Shu, R. Mou, J. Lombardi, G. Prestwich, M. Rafailovich, R. Clark, *Biomacromolecules* **2005**, *6*, 2857.
[31] E. Kim, J. Oh, I. Ahn, K. Park, J. Jang, *Biomaterials* **2011**, *32*, 8654.